

Spatial genomics of AAV vectors reveals mechanism of transcriptional crosstalk that enables targeted delivery of large genetic cargo

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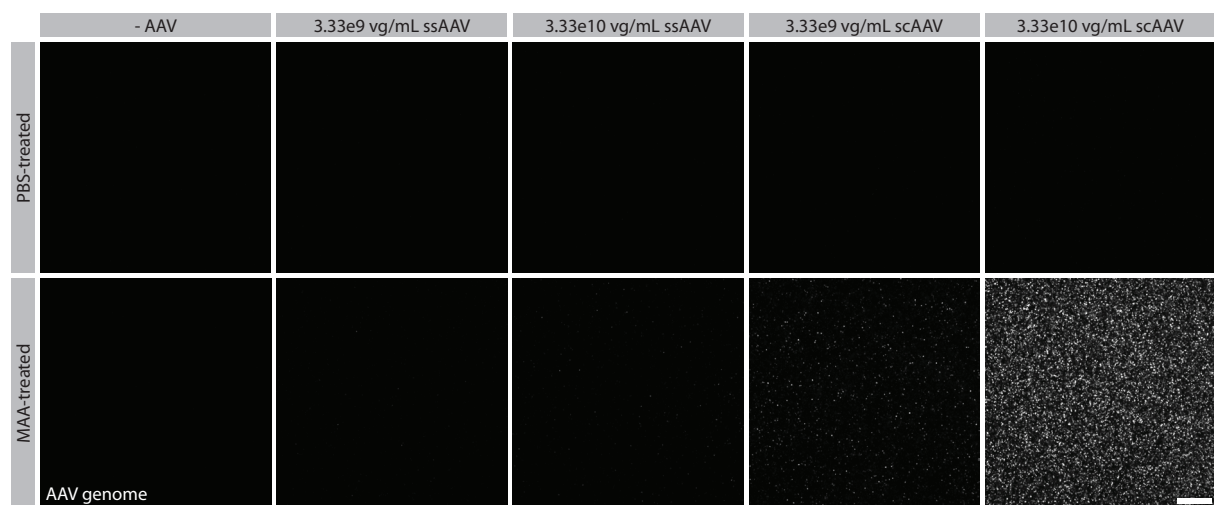
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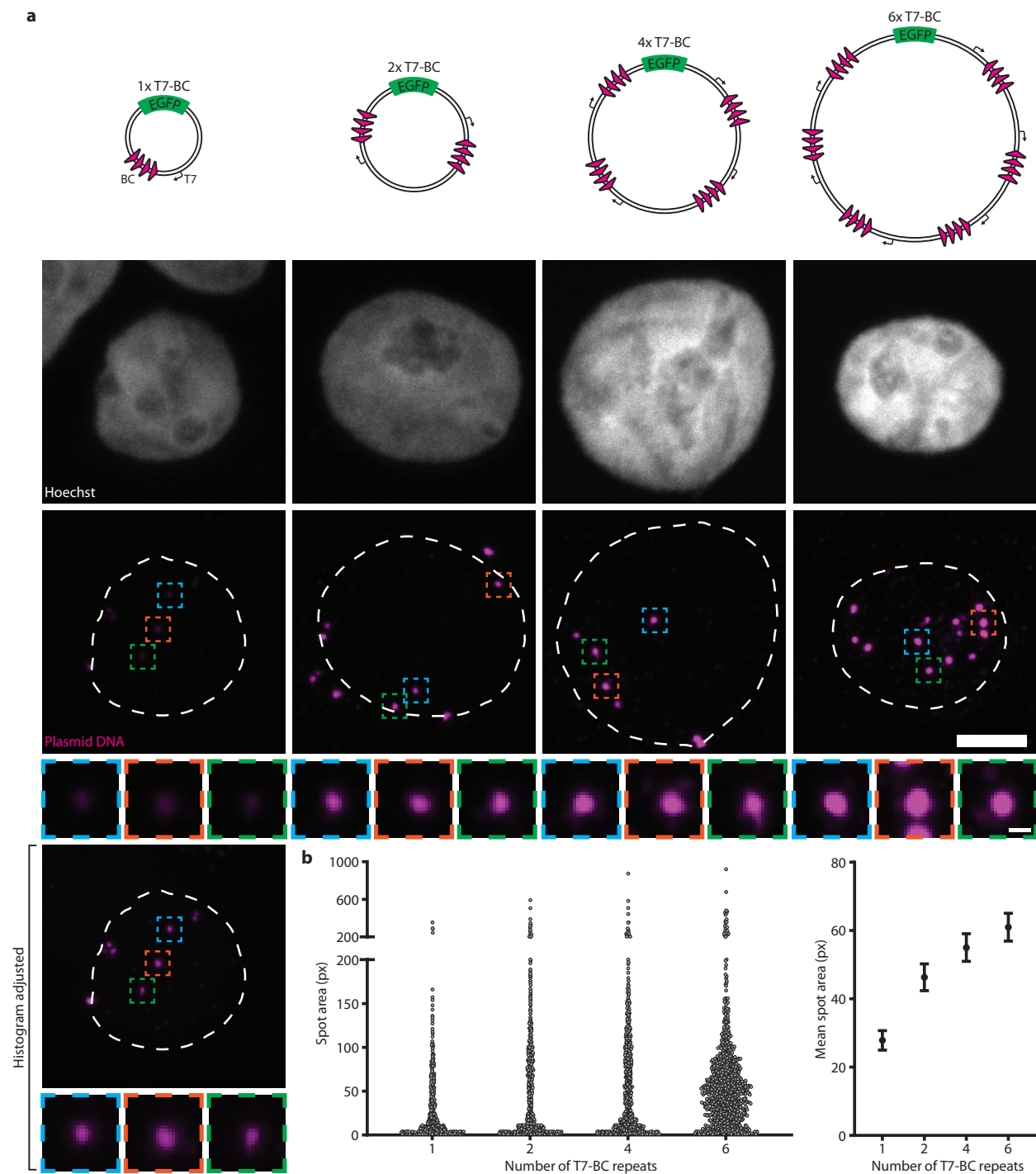
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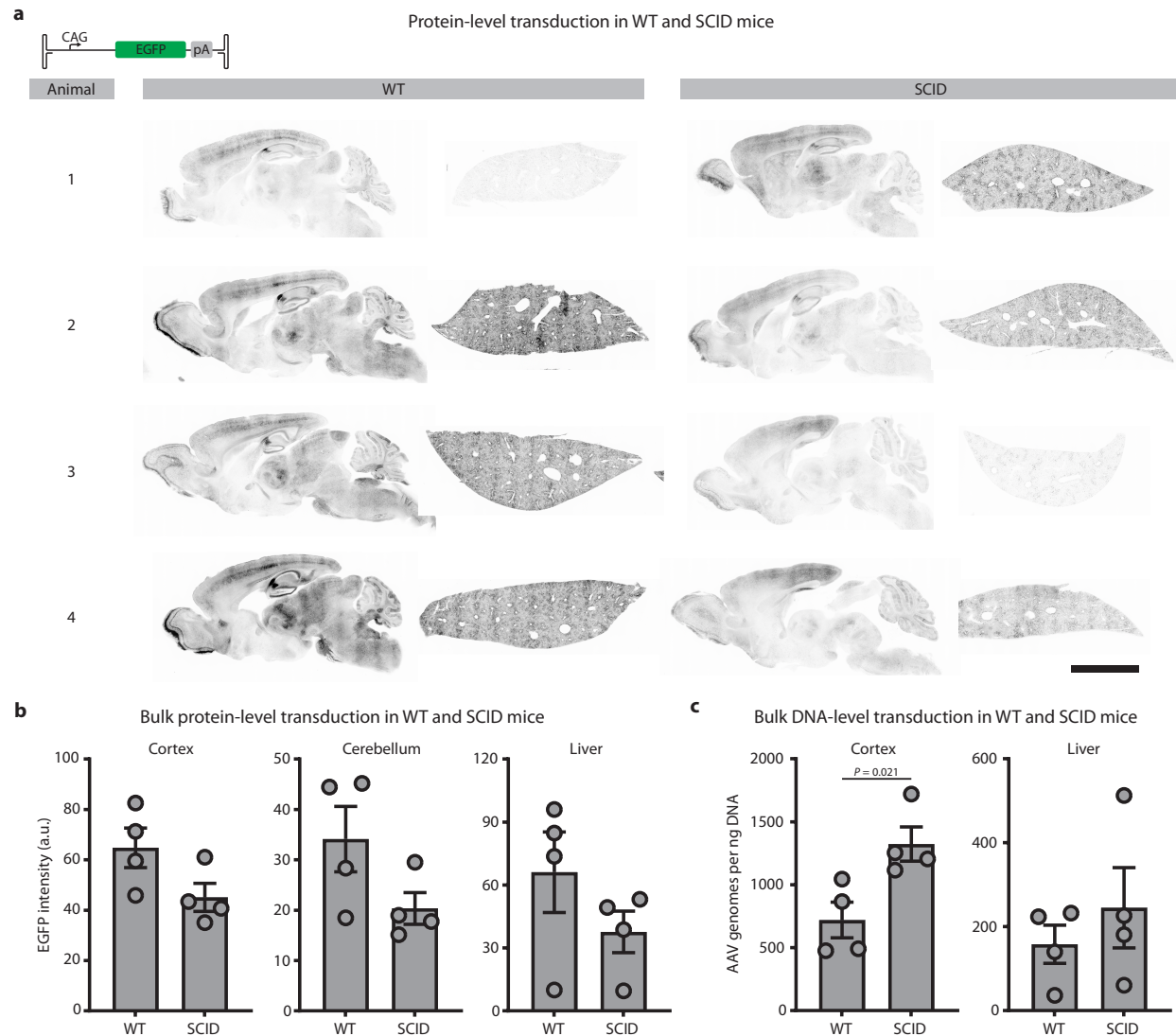
Supplementary Data Figure 1. Methanol and acetic acid fixation is sufficient to denature the AAV capsid, enabling genome detection by AAV-Zombie. To investigate whether processing of the AAV genome by the host cell was necessary for detection by AAV-Zombie, we devised a cell-free system. Single-stranded and self-complementary genomes were packaged into AAV-DJ, and then embedded in high-concentration Matrigel. Following gelation, the samples were treated with ice-cold 1x PBS (top row) or MAA (bottom row). MAA treatment resulted in an increase in Zombie signal from scAAV samples, as compared to PBS controls, suggesting that MAA treatment can denature the capsid, releasing the AAV genome. Scale bar = 20 μ m.



Supplementary Data Figure 2. Relationship between number of T7-barcode repeats and spot area.

a, Plasmids with increasing numbers of T7-barcode (T7-BC) repeats were constructed and transfected at equimolar amounts into HEK293T cells. Zombie was then used to detect the individual plasmids. Scale bar = 20 μm for larger images, 2 μm for insets. **b**, Quantification of spot area as a function of number of

T7-BC repeats. Plasmids with more T7-BC repeats yielded larger spots. $n = 706$ (1 T7-BC repeat), 932 (2 T7-BC repeats), 1052 (4 T7-BC repeats), 999 (6 T7-BC repeats) spots per condition. Points represent mean and bars are 95% confidence interval.



Supplementary Data Figure 3. AAV transduction of wildtype and SCID mice. **a**, Wildtype and SCID C57BL/6J animals were transduced with 3e11 vg of AAV-PHP.eB packaging a CAG-EGFP reporter, and tissue was collected 4 weeks later. Representative sagittal brain (left) and liver (right) sections are shown. Scale bar = 5 mm. **b**, Quantification of bulk protein in cortex (left), cerebellum (middle), and liver (right) of WT and SCID animals, assessed by mean EGFP intensity. **c**, Quantification of bulk viral DNA in WT and SCID cortex and liver, assayed through digital droplet PCR. SmaI digests and KpnI-HF/SpeI-HF digests yielded similar results; results from SmaI-digested samples are shown. For (**b**) and (**c**), statistical significance was determined using unpaired t-tests. $n = 4$ animals per genotype. Bars are mean $\pm$ s.e.m.

Supplementary Video 1. Representative videos of narrowing beam crossing performance for animals in ubiquitous SaCas9 condition. For display purposes, videos are trimmed to show crossing of 2.5 cm wide segment of beam. The entire length of beam was used for data analysis. Videos show the same animals pre-injection and 4 weeks post-injection.

Supplementary Video 2. Representative videos of narrowing beam crossing performance for animals in crosstalk-mediated PC-specific SaCas9 condition. For display purposes, videos are trimmed to show crossing of 2.5 cm wide segment of beam. The entire length of beam was used for data analysis. Videos show the same animals pre-injection and 4 weeks post-injection.

Supplementary Table 1. Indications where transcriptional crosstalk may be therapeutically beneficial for high or cell type-specific expression.

Disorder	Human Gene (bp)	Target Cell Type	Targeting Rationale	References
Alpha-1 antitrypsin deficiency	SERPINA1 (1254)	Lung, Liver*	Mutations in SERPINA1 cause accumulation in the liver and underexpression in the lung. Enhancing lung expression while minimizing liver expression would be beneficial. Though this transcript is relatively short, crosstalk-mediated enhancement may be necessary due to potential need for several regulatory motifs on the genome.	1,2
CDKL5 Deficiency Disorder	CDKL5 (3427)	Cerebral Cortex, Cerebellum	CDKL5 is primarily expressed in neurons, both excitatory and inhibitory, and highly expressed in early development. Inclusion of a temporally sensitive enhancer element could promote high expression during the critical period, while maintaining a lower baseline expression level.	3
Cornelia De Lange Syndrome	BRD4 (4089), SMC1A (3969)	Cerebral Cortex Neurons, Epithelial Cells, Smooth Muscle and Enteric Nervous System	Brd4 overexpression is linked to cancer progression and inflammatory dysregulation, emphasizing the incentive to restrict unnecessary expression in off-target cell types. Smc1a overexpression can dysregulate chromosomal segregation in mitosis and meiosis.	4–7
Cystic Fibrosis	CFTR (4440)	Secretory Epithelia in Lung, Pancreas	The coding sequence length for CFTR limits promoter and regulatory element choices in a single AAV genome. Additionally, CFTR is not expressed in the muscle, heart, neurons or blood cells. Expressing chloride channels in non-target cell types could perturb homeostatic processes	8
Duchenne Muscular Dystrophy	mini- or micro-dystrophin (varies)	Skeletal Muscle	Dystrophin, including miniaturized constructs, have long sequences, which limit the inclusion of gene regulatory elements.	9
Osteogenesis Imperfecta; Ehlers Danlos Syndrome	COL1A1 (4392)	FSP+ mesenchymal cells, fibroblasts	Subpopulations of COL1A1 producing cells are more specifically responsible for the OI phenotype. Aberrant overexpression of COL1A1 may lead to extensive tissue fibrosis and complications, particularly in the vascular system.	10,11
Pyruvate Carboxylase Deficiency	PC (3534)	Hepatocytes, Astrocytes	Overexpression or off target expression could lead to metabolic imbalances, including overaccumulation of downstream metabolites	12
Spinocerebellar Ataxia 2; Parkinsons Disease	Atxn2 (3948)	Purkinje Cells, Basal Ganglia Neurons	Different cell populations can be targeted for different pathologies using the same transgene; Purkinje cells for SCA2, but to Basal Ganglia for PD	13,14
Spinocerebellar Ataxia 44	Grm1 (3585)	Purkinje Cells	Some patients display both cerebellar and cortical involvement, but Grm1 is expressed at much higher levels in Purkinje cells.	15,16
SYNGAP1-related intellectual disability	SYNGAP1 (3879)	Hippocampal neurons, Cerebellar granule cells	SYNGAP1 is not expressed in non-neuronal cell types in the CNS, and its sequence length limits inclusion of additional regulatory elements in AAV genome.	17
Wilson's Disease	ATP7B (4410)	Hepatocytes, Basal Ganglia Neurons, Cornea	Hepatocytes express ATP7B at a much higher level than other tissues, thus enhancement may be desirable there while not overexpressing in other tissues	18

Supplementary Table 1 References

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